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SUNY Upstate Medical University
Committee for the Humane Use of Animals

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For CHUA use only

CHUA # 713
Addendum # 713A
Principal Investigator Meguid
Date Received: 7-18-01
Date Approved: 7-24-01

ADDENDUM TO AN APPROVED PROTOCOL

Please attach a typed, **detailed** description of all changes from the original protocol and the explanation for these changes. The appropriate types of changes include (but are not limited to) any of the following: additional test substances administered, changes in anesthesia, changes in data collection, changes in species or strain, need for additional animals, etc. Complex changes in experimental procedure or multiple departures from the original protocol may require submission of an entirely new protocol. Contact the CHUA office for guidance.

As mandated by the Animal Welfare Act, 9 CFR Ch. 1: If any of the following items **will be altered from the original protocol** by the proposed changes, those changes must be addressed within the addendum.

Species/strain involved and source.

Approximate number of animals needed for this addendum.

Total number of animals requested for this CHUA.

Changes in groups, numbers/group, etc.

Rationale for species change and/or the numbers requested.

Changes in proposed use of animals (describe completely).

Changes in anesthesia or other methods of minimizing pain and discomfort.

Changes in euthanasia methods.

Change in the pain category that requires consideration of alternatives to minimize pain and suffering.

Please ensure that the attached addendum includes the CHUA number, date, printed name and signature of the principal investigator.

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Approvals
(Initial & date)

CHUA Chairperson [Signature] Date 7-19-01
Veterinarian [Signature] Date 7-24-01
Other CHUA Member _____ Date _____

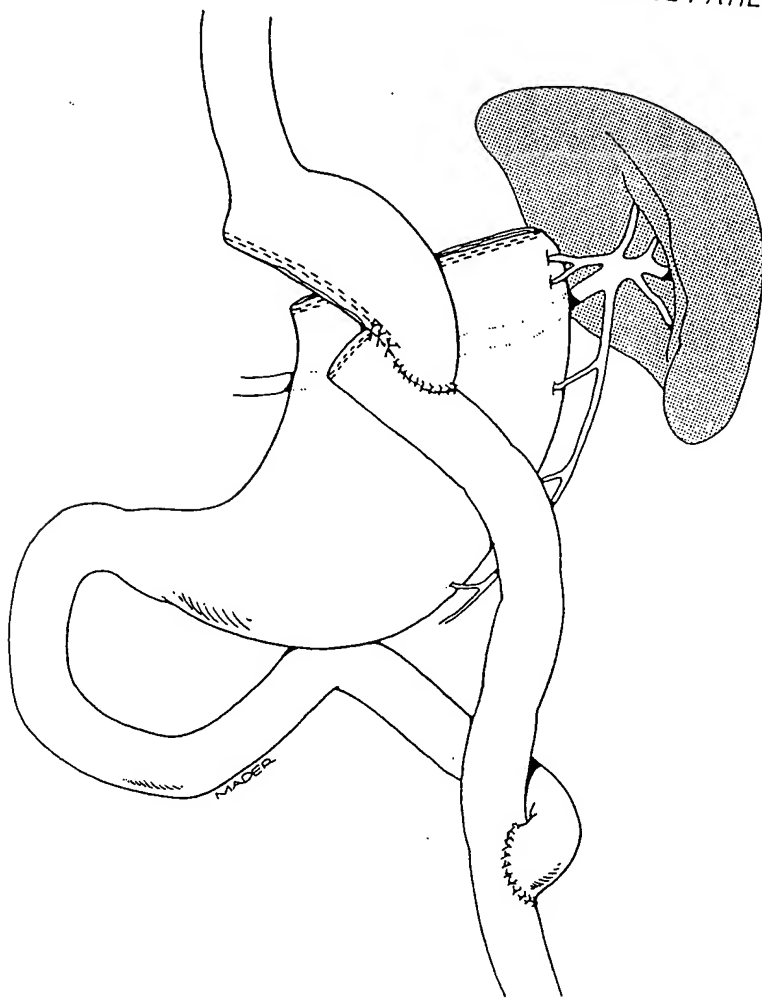


FIGURE 12.5. Authors' version of divided gastric bypass with Roux-en-Y.

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CHUA# 713
Addendum#
Principal Investigator
Date Received:
Date Approved:

Oct. 12, 2001

1. Species/strain involved and source: Obese Zucker (fa/fa) rats. Harlan Teklad.
2. Approximate number of animals needed for this addendum: 24⁺
3. Changes in groups, numbers/ group, etc.

8 obese rats will be used as study (gastric bypass operation); 8 obese rats will be used as pair fed sham control and 8 obese rats will be used as ad libitum control.

4. Rationale for species change and or the numbers requested.

The rationale for using 24 obese Zucker rats is:

- i). As showed in Figure 1, it is likely that the reduced food intake and weight loss after gastric stapling may be due to acute effect of operation. To verify this point and to eliminate this issue it is important to test the effects of gastric-bypass after 21-30 days.
- ii) It is also needed to add a pair fed control group for this experiment to verify effects on mRNA (see figure 2), to observe long-term (21 days) effect after gastric bypass operation on body weight loss and expression of gastric mRNA of ghrelin.

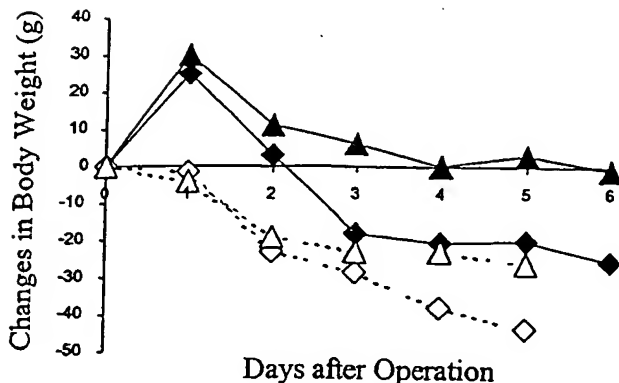


Fig.i. Changes in body weight after gastric-bypass operation in Zucker rats. Both obese and lean rats lost body weight after gastric-bypass operation and rats in sham operation group lost weight after pair-fed food intake.

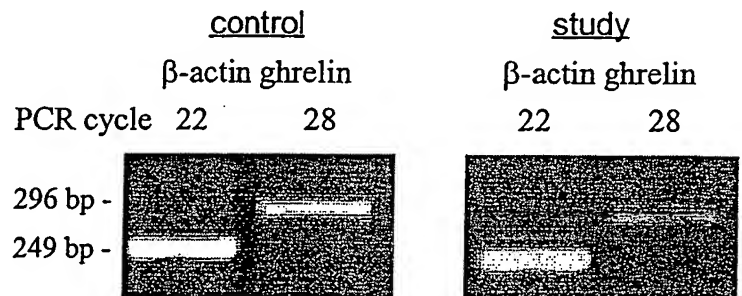


Fig. 8. RT-PCR for Ghrelin mRNA from stomach one week after gastric bypass operation in SD rats. The bands of 296 and 249 base pairs corresponded to the expected fragments of ghrelin and β-actin, respectively.

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CHUA # 713
Addendum # 713C
Principal Investigator M. Quinn
Date Received: 12-11-01
Date Approved: 1-9-02

ADDENDUM TO AN APPROVED PROTOCOL

Please attach a typed, **detailed** description of all changes from the original protocol and the explanation for these changes. The appropriate types of changes include (but are not limited to) any of the following: additional test substances administered, changes in anesthesia, changes in data collection, changes in species or strain, need for additional animals, etc. Complex changes in experimental procedure or multiple departures from the original protocol may require submission of an entirely new protocol. Contact the CHUA office for guidance.

As mandated by the Animal Welfare Act, 9 CFR Ch. 1: If any of the following items **will be altered from the original protocol** by the proposed changes, those changes must be addressed within the addendum.

Species/strain involved and source.

Approximate number of animals needed for this addendum.

Total number of animals requested for this CHUA.

Changes in groups, numbers/group, etc.

Rationale for species change and/or the numbers requested.

Changes in proposed use of animals (describe completely).

Changes in anesthesia or other methods of minimizing pain and discomfort.

Changes in euthanasia methods.

Change in the pain category that requires consideration of alternatives to minimize pain and suffering.

Please ensure that the attached addendum includes the CHUA number, date, printed name and signature of the principal investigator.

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Approvals
(Initial & date)

CHUA Chairperson

Veterinarian

Other CHUA Member

Date

Date

Date

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CHUA # 713
Addendum # 713D
Principal Investigator Meguid
Date Received: 2-25-02 Rev.
Date Approved: 3-12-02

ADDENDUM TO AN APPROVED PROTOCOL

Please attach a typed, **detailed** description of all changes from the original protocol and the explanation for these changes. The appropriate types of changes include (but are not limited to) any of the following: additional test substances administered, changes in anesthesia, changes in data collection, changes in species or strain, need for additional animals, etc. Complex changes in experimental procedure or multiple departures from the original protocol may require submission of an entirely new protocol. Contact the CHUA office for guidance.

As mandated by the Animal Welfare Act, 9 CFR Ch. 1: If the following items will be altered from the original protocol, please address each in the addendum regarding the proposed changes. Any non-applicable items must be indicated by N/A.

1. Species/strain involved and source.
2. Approximate number of animals needed for this addendum.
3. Total number of animals requested for this CHUA.
4. Changes in groups, numbers/group, etc.
5. Rationale for species change and/or the numbers requested.
6. Changes in proposed use of animals (describe completely).
7. Changes in anesthesia or other methods of minimizing pain and discomfort.
8. Changes in euthanasia methods.
9. Change in the pain category that requires consideration of alternatives to minimize pain and suffering.

Please ensure that the attached addendum includes the CHUA number, date, printed name and signature of the principal investigator.

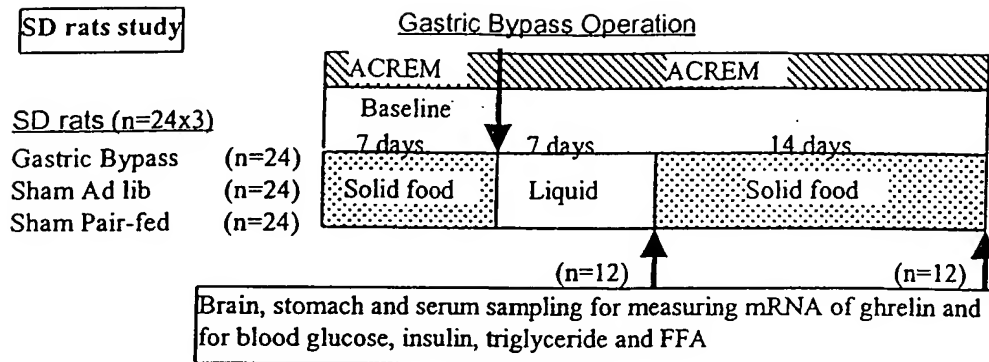
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Approvals
(Initial & date)

CHUA Chairperson
Veterinarian
Other CHUA Member

Date 3/2/02
Date 3/12/02
Date _____

Figure 1: Experiment #1

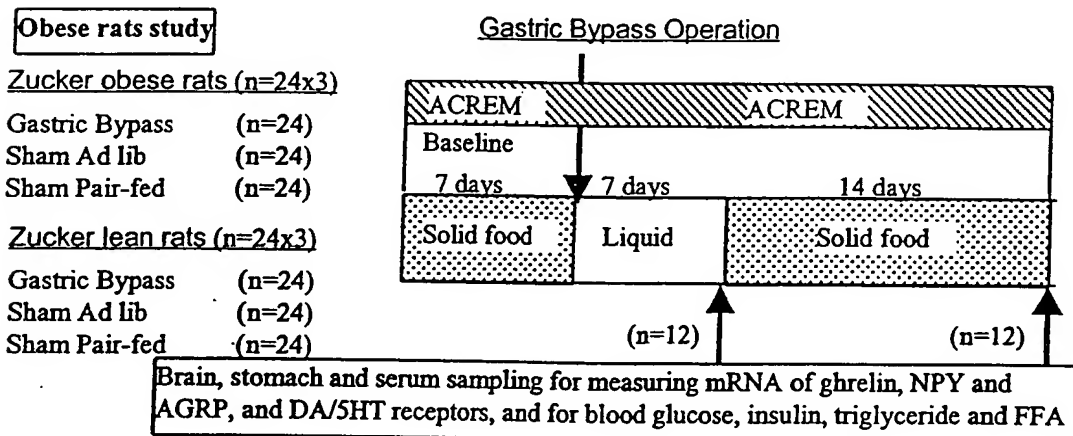


EXP #2-Zucker rats study (in 2002): The purpose of this study is to investigate short- and long-term effects of gastric bypass operation on the feeding pattern, body weight and carcass composition changes.

In this experiment, the mRNA of ghrelin, NPY and AGRP, and DA/5HT receptors will be analyzed with molecular biology method. The mRNA will be extracted from hypothalamus homogenates for measuring the expression of ghrelin, NPY, AGRP and of dopamine D1, D2, serotonin 5ht1b or 5ht2c receptors with RT-PCR method.

There are 72 Zucker obese and 72 lean rats in this study, rats are divided into 6 groups, each group has 24 obese or 24 lean Zucker rats, see Fig. 2

Figure 2: Experiment #2



EXP #3-Histological study (in 2003): The purpose of this study is to confirm the specific sites and the amount of peptide and its gene expression, by using immunohistochemistry and in situ hybridization for: ghrelin, NPY, AGRP and dopamine D1, D2, serotonin 5HT1B or 5HT2C receptors in hypothalamus, and ghrelin in stomach in Zucker rats. This will give the possibility to quantify the results of immunocytochemical reaction, and as a result changes in the receptor activity after operation in different hypothalamic structures. Immunofluorescent doublelabeling with different labeling of secondary antibodies will be performed to analyze the possible colocalization of serotonin and dopamine receptors on the same neurons, and thus

We hypothesize that gastric stapling creates a small gastric pouch inducing early satiety by reducing the gastric peptide ghrelin. This inhibits gastric vagal afferents that relay signals to the hypothalamus, which stimulate aminergic (5HT) and inhibit peptidergic (NPY/AGRP) to decrease food intake. Based on the result of our previous experiments, we had developed a rat Gastric Bypass, Roux-en-Y (GB) model and observed decreased food intake and weight loss. This would enable us to continue our obesity study and explore the peripheral and central mechanisms whereby this operation induces gradual weight loss.

5. Changes in proposed use of animals. None.

This is an addendum to original protocol, in order to continue the obesity study.

6. Changes in anesthesia or other methods of minimizing pain and discomfort.

None.

7. Changes in euthanasia methods.

None. Same as original protocol.

8. Change in the pain category that requires consideration of alternative to minimizing pain and suffering.

None. Same as original protocol

Michael Meguid MD PhD

- iv) Method for the model of DIO rats:

Sprague-Dawley male rat (specifically from Charles River Laboratories), 3 weeks of age are kept on Purina lab chow (no. 5008) and water ad libitum for 1 week, food intake and body weight is measured. Then all rats are switched to a HE diet ad libitum for 5-6 weeks. This diet is composed of 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow (no.5001, Research Diets) and

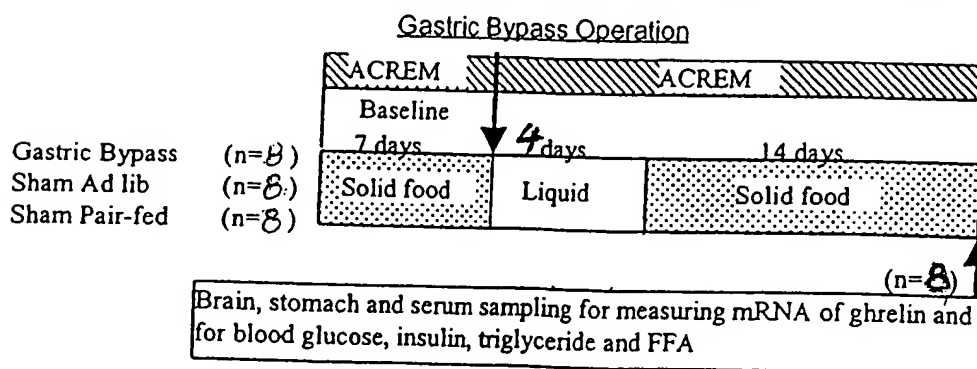
contains

4.47 kcal/g, with 21% of the metabolizable energy content as protein, 31% as fat, and 48% as carbohydrate, 50% of which is sucrose. This diet is provided by Research Diets, NJ (no. D12266B). After 5-6 weeks on the HE diet, rats become fat (~100%), and are designated as diet-induced obesity rat (DIO).

Reference:

- (1) Levin BE, Dunn-Meynell AA. Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 2002; 282(1): R46-54
- (2) Levin BE, Keeseey RE. Defense of differing body weight set points in diet-induced obese and resistant rats. *Am J Physiol*. 1998 Feb;274(2 Pt 2):R412-9
- (3) Levin BE, Dunn-Meynell AA, Balkan B, Keeseey RE. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *Am J Physiol*. 1997 Aug; 273(2 Pt 2):R725-30.

2. Species/strain involved and source.
3-week old weanling Sprague Dawley rat. Charles River Laboratories.
3. Approximate number of animals needed for this addendum. 32
4. Total number of animals requested for this CHUA. 540
5. Changes in groups, numbers/group, etc.
None.
6. Changes in proposed use of animals (describe completely).
None. This is only an addendum to original protocol, whereby we will reproduce the Roux-en-Y gastric bypass. The groups and surgery are same as explained in the original protocol and Addendum of Mar 7, 2002. 24 rats will be used in this experiment. For assuring to get the most obese rats for this study, we apply for 32 rats to feed them with high energy diet instead of only 24.
Experiment designed: 24 Diet-induced obese rats will be divided into three group: Gastric bypass (GB, n=8), Sham Ad lib (SA, n=8) and Sham Pair-fed (SP, n=8). 8 left rats will be used for other protocol.



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